Supplementary data

Antiviral adsorption activity of porous silicon nanoparticles against different pathogenic human viruses

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Virus purification process for ELISA experiments

Ten-day-old embryonated chicken eggs were inoculated with 10^2 EID (embryo infective dose) of viruses, incubated at 36°C, monitored and cooled immediately after 48 hours of incubation. The study design was approved by the Ethics Committee of the Chumakov Institute of Poliomyelitis and Viral Encephalitides, Moscow, Russia (Approval #4 from 2 December 2014).

The virus-containing cultural or allantoic fluid was clarified by centrifugation at 5200 rpm within 15 minutes. Supernatant was centrifugated at 23000 rpm 90 minutes in an SW-27.1 rotor at 4^0C. The virus-containing fluid was layered on 4 ml of 20% sucrose in phosphate buffered saline (PBS, pH7,4). The concentrated virus was resuspended in 2 ml of the same buffer solution.

The content of the virus in the suspension was determined in the hemagglutination assay and the protein content, via the Bradford method [1s]. The hemagglutination assay is based on the binding (agglutination) of red blood cells (erythrocytes), which occurs after a certain concentration of viruses have attached to the surface of the red blood cells. The ability of viruses to agglutinate erythrocytes is associated with their infectivity; therefore, this method can be used to quantify the total amount of viral particles. By successively diluting the virus suspension and adding a standard number of erythrocytes, the presence of viral particles (in HAU/ml) was assessed. According to the data obtained, the concentration of the viral suspension of 1 mg/ml corresponded to 215 HAU/ml.

[1s] M. M. Bradford, A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. Analytical biochemistry, 72(1-2) (1976), 248-254.

Virus purification process for in vitro experiments

The cells in the late logarithmic phase were washed twice with RPMI-1640 medium and virus-containing preparations (virus diluted in medium) were added in a dose of 1 $TCID_{50}$ per cell (infection dose). After 1-h incubation at 37° C, the cells were washed once with RPMI-1640 medium and maintenance medium was added (RPMI-1640 supplemented with 1% fetal calf serum). Then, the infected cultures were incubated at 37° C. The time of passaging was limited by the cytopathic effect (CPE) of the virus. On the next day after infection, syncytia in the culture were evaluated. On days 2-3, the number of syncytia significantly increased and the number of dead cells sometimes exceeded 70 %. The percent of dead cells was evaluated by vital staining with 1% trypan blue in a Goryaev's chamber. After attaining the cytopathic effect (cell death >70%), the obtained virus-containing samples (suspension of viruses with cell detritus) were clarified by centrifugation (5000 rpm, 30 min). The clarified virus-containing preparations were layered on sucrose gradient (20-30% sucrose cushion) and centrifuged (Beckman Coulter

ultracentrifuge; rotor 50.2 Ti; 160,000 g, 1.5 h, 5° C). The precipitate was resuspended in PBS or culture medium prepared ex tempore using the same centrifugation regimen (for removal of conglomerates). The purified viruses were used for further study. The virus containing suspensions were stored at 4° C for 2 weeks. Longer storage was performed at -80° C or in liquid nitrogen.

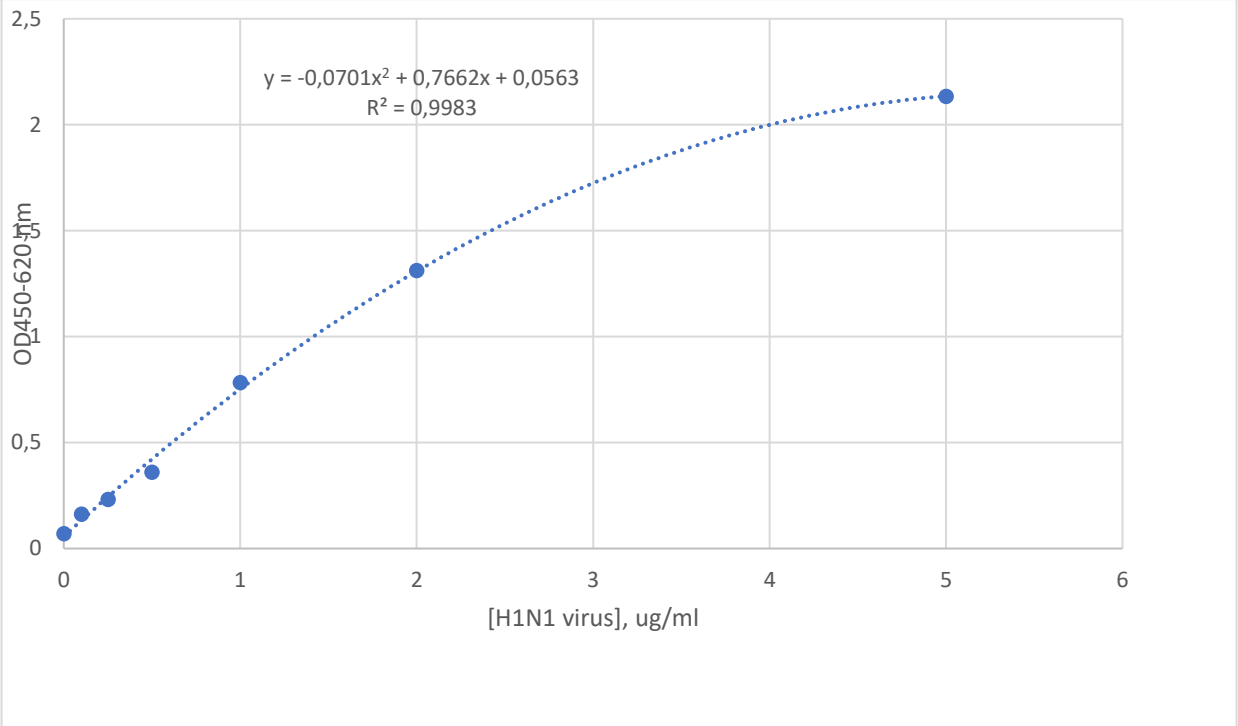

Fig. S1. Typical calibration curve to detect H1N1 virus in solution by *ELISA*.

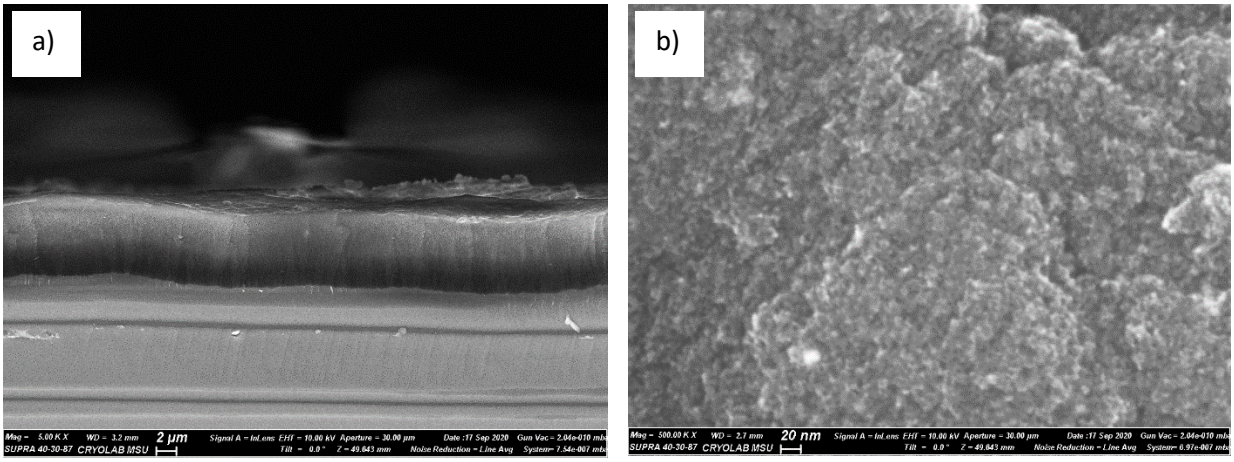

Fig. S2. SEM cross-section microphotograph of PSi film (a); SEM cross-section microphotograph of PSi film taken at high magnification (b).

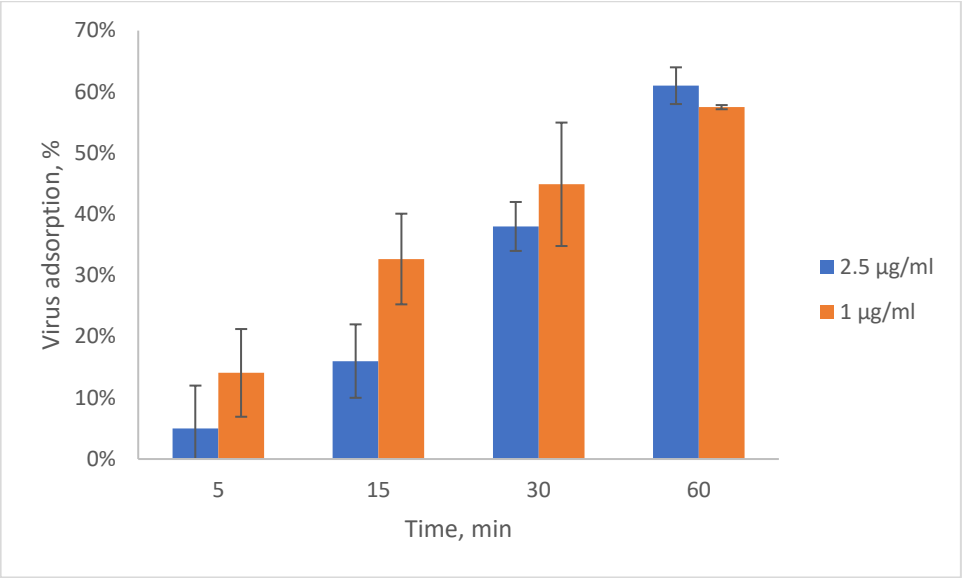

Fig. S3. *ELISA* analysis of antiviral adsorption activity of PSi NPs to the H1N1 Influenza virus for different incubation time: 5, 15, 30 and 60 min. The final concentration of nanoparticles was 0,5 mg/ml, the concentration of the virus was 1 or 2.5 µg/ml.

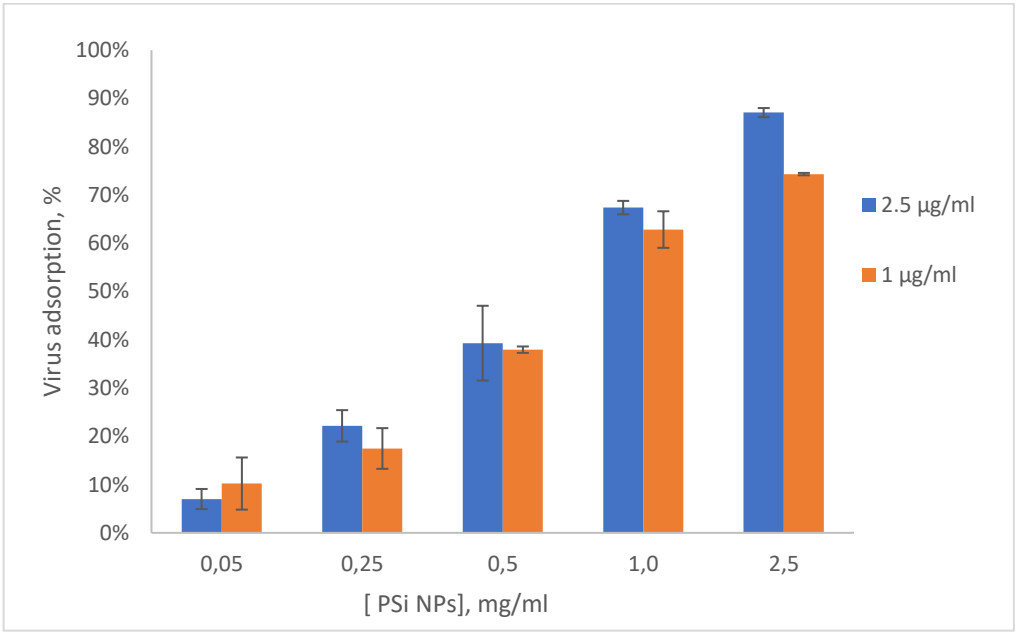

Fig. S4. *ELISA* analysis of antiviral adsorption activity of PSi NPs, taken at concentrations of 0.05, 0.25, 0.5, 1 and 2.5 mg/ml to the H1N1 Influenza virus. The final virus concentration was 1 or 2.5 µg/ml, the incubation time was 20 min.

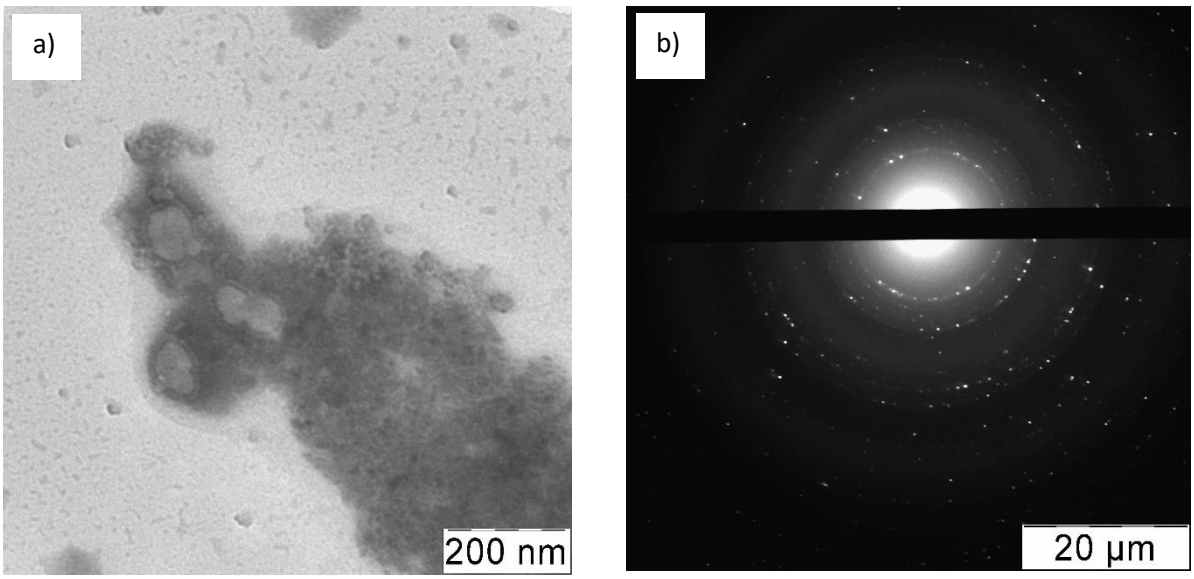

Fig. S5. TEM image of Influenza virions trapped in the mesh of PSi NPs (a); Pattern of the PSi NPs electron diffraction, obtained from the left image (b).